1. Introduction
Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerase offers extreme performance for all major PCR applications. Incorporating an exciting technology, Phusion DNA Polymerase brings together a novel Pyrococcus-like enzyme with a processivity-enhancing domain. The Phusion DNA Polymerase generates long templates with an accuracy and speed previously unattainable with a single enzyme, even on the most difficult templates. The extreme fidelity makes Phusion DNA Polymerase a superior choice for cloning. Using a ladd-based method modified from previous studies, the error rate of Phusion DNA Polymerase in Phusion HF Buffer is determined to be 4.4 × 10⁻¹, which is approximately 50-fold lower than that of Thermus aquaticus DNA polymerase, and 6-fold lower than that of Pyrococcus furiosus DNA polymerase.

Phusion DNA Polymerase possesses the following activities: 5 ‒ 3 DNA polymerase activity and 3 ‒ 5 exonuclease activity. It generates blunt ends in the amplification products. The polymerase is also capable of amplifying long amplicons such as the 7.5 kb genomic and 20 kb α DNA used in Thermo Fisher Scientific quality control assays. The 5X Phusion Green HF Buffer and 5X Phusion Green GC Buffer include a density reagent and two tracking dyes for excitation, we recommend using the colorless 5X Phusion HF Buffer (F-518) or 5X Phusion GC Buffer (F-519) or excitation, we recommend using the colorless 5X Phusion HF Buffer (F-518) or 5X Phusion GC Buffer (F-519) or purifying the PCR product prior to analysis.

2. Important Notes
• Use 98 °C for denaturation (see sections 5.1 and 5.2).
• The annealing rules are different from many common DNA polymerases (such as Taq DNA polymerases). Read section 5.3 carefully.
• Use 15–30 s/kb for extension. Do not exceed 1 min/kb (see section 5.4).
• Use Phusion DNA Polymerase at 0.5–1.0 U per 50 μL reaction volume. Do not exceed 2 U/50 μL (see section 4.1).
• Use 200 μM of each dNTP. Do not use dUTP (see section 4.3).
• Phusion DNA Polymerases produce blunt end DNA products.

3. Guidelines for using Phusion DNA Polymerase
Carefully mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery. PCR reactions should be set up on ice. Prepare a master mix for the appropriate number of samples to be amplified. Phusion DNA Polymerase should be pipetted carefully and gently as the high glycerol content (50%) in the storage buffer may otherwise lead to pipetting errors.

It is critical that the Phusion DNA Polymerase is the last component added to the PCR mixture, since the enzyme exhibits 3 → 5 exonuclease activity that can degrade primers in the absence of dNTPs. Due to the nature of Phusion DNA Polymerase, the optimal reaction conditions can be different from PCR protocols for standard DNA polymerases. Due to the high salt concentration in the reaction buffer, Phusion DNA Polymerase tends to work better at elevated denaturation and annealing temperatures. Please pay special attention to the conditions listed in section 5 when running your reactions. Following the guidelines will ensure optimal enzyme performance.

1. For Research Use Only. Not for use in diagnostic procedures.

www.thermofisher.com
For Research Use Only. Not for use in diagnostic procedures.
If high DMSO concentration is used, the annealing temperature must be decreased, as DMSO affects the melting point of the primers. It has been reported that 10% DMSO decreases the annealing temperature by 5.5–8 °C.

5. Notes on cycling conditions

5.1. Initial denaturation

Denaturation should be performed at 98 °C. Due to the high thermostability of Phusion DNA Polymerase even higher denaturation temperatures can be used. We recommend a 30-second initial denaturation at 98 °C for most templates. Some templates may require longer initial denaturation time and the length of the initial denaturation time can be extended up to 3 minutes.

5.2. Denaturation

Keep the denaturation time as short as possible. Usually 5–10 seconds at 98 °C is enough for most templates. Note: The denaturation time and temperature may vary depending on the ramp rate and temperature control mode of the cycler.

5.3. Primer annealing

The optimal annealing temperature for Phusion DNA Polymerase may differ significantly from that of Taq-based polymerases. Always use the Tm calculator and instructions on our website (www.thermofisher.com/stccalculator) to determine the Tm values of primers and optimal annealing temperature. If necessary, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination. The annealing gradient should extend up to the extension temperature (two-step PCR).

A 2-step protocol is recommended when primer Tm values are at least 69 °C (≥ 20 nt) or 72 °C (≤ 20 nt) when calculated with our Tm calculator. In the 2-step protocol the combined annealing/extension step should be performed at 72 °C even when the primer Tm is ≥ 72 °C.

5.4. Extension

The extension should be performed at 72 °C. Extension time depends on amplicon length and complexity. For low complexity DNA (e.g., plasmid, lambda or BAC DNA) use an extension time of 15 seconds per 1 kb. For high complexity genomic DNA 30 seconds per 1 kb is recommended. For some cDNA templates, the extension time can be increased up to 40 seconds per 1 kb to obtain optimal results.

6. Recommendations for cloning

When cloning fragments amplified with Phusion DNA Polymerase, blunt end cloning is recommended. If TA cloning is required, it can be performed by adding A overhangs to the blunt PCR product with Thermo Scientific™ Taq DNA Polymerase (#EP0402), for example.

Incubate purified PCR product with 1x Taq buffer, 2.5 mM MgCl₂, 0.2 mM dATP and 1 U Taq DNA polymerase in 10 μL reaction mixture up to 30 min at 72 °C. Before adding the overhangs it is very important to remove all the Phusion DNA Polymerase by carefully purifying the PCR product, for example using Thermo Scientific™ GeneJET™ PCR Purification Kit (#K0701). Any remaining Phusion DNA Polymerase will degrade the A overhangs, creating blunt ends again.

7. Troubleshooting

No product at all or low yield

- Repeat and make sure that there are no pipetting errors.
- Use fresh high quality dNTPs.
- Do not use dNTP mix or primers that contain dUTP or dITP.
- Sample concentration may be too low. Use more template.
- Template DNA may be damaged. Use carefully purified template and make sure template is not fragmented.
- Increase extension time.
- Increase the number of cycles.
- Optimize annealing temperature.

Optimize enzyme concentration.
- Titrate DMSO (2–8 %) in the reaction (see section 4.5).
- Denaturation temperature may be too low. Optimal denaturation temperature for most templates is 98 °C or higher.
- Optimize the denaturation time.
- Check the purity and concentration of the primers.
- Check primer design.
- Try using the alternative GC Buffer (see section 4.2).

Non-specific products - High molecular weight smear

- Decrease enzyme concentration (see section 4.1).
- Decrease extension time (see section 5.4).
- Reduce the total number of cycles.
- Increase annealing temperature or try 2-step protocol (see section 5.3)
- Vary denaturation temperature (see section 5.2).
- Optimize Mg²⁺ concentration.
- Decrease primer concentration.

Non-specific products - Low molecular weight discrete bands

- Increase annealing temperature (see section 5.3).
- Decrease extension time (see section 5.4).
- Decrease enzyme concentration.
- Optimize Mg²⁺ concentration.
- Titrate template amount.
- Decrease primer concentration.
- Design new primers.

8. Component specifications

8.1. Phusion High-Fidelity DNA Polymerase (F-530)

Thermostable Phusion DNA Polymerase is purified from an E.coli strain expressing the cloned Phusion DNA Polymerase gene. Phusion DNA Polymerase possesses the following activities: 5 → 3 DNA polymerase activity and 3 → 5 exonuclease activity.

8.2. 5X Phusion Green HF Buffer (F-538)

The 5X Phusion Green HF Buffer contains 7.5 mM MgCl₂, which provides 1.5 mM MgCl₂ in final reaction conditions.

8.3. 5X Phusion Green GC Buffer (F-539)

The 5X Phusion Green GC Buffer contains 7.5 mM MgCl₂, which provides 1.5 mM MgCl₂ in final reaction conditions.

8.4. 50 mM MgCl₂ Solution (F-510MG)

Both Phusion Buffers supply 1.5 mM MgCl₂ at final reaction conditions. If higher MgCl₂ concentrations are desired, use 50 mM MgCl₂ solution to increase the MgCl₂ titer. Using the following equation, you can calculate the volume of 50 mM MgCl₂ needed to attain the final MgCl₂ concentration: [desired mM Mg²⁺] × [1.5 mM] = μL to add to a 50 μL reaction. For example, to increase the MgCl₂ concentration to 2.0 mM, add 0.5 μL of the 50 mM MgCl₂ solution. Because the PCR reactions can be quite sensitive to changes in the MgCl₂ concentration, it is recommended that the 50 mM MgCl₂ stock solution is diluted 1:5 (to 10 mM) to minimize pipetting errors.

8.5. Dimethyl sulfoxide DMSO, 100% (F-519)

Note: The freezing point of DMSO is 18 °C, so it does not melt at 4 °C.

9. References


CERTIFICATE OF ANALYSIS

Endonuclease contamination assay

No endonuclease activity was observed after incubation of DNA polymerase with supercoiled plasmid DNA.

DNA amplification assay

Performance in PCR is tested by the amplification of a 7.5 kb fragment of genomic DNA and a 20 kb fragment of lambda DNA.

Quality authorized by: Jurgita Zilinskiene